Characterization of CRISPR-Cas9 Induced SAUR19 Family Mutants in Arabidopsis.

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Background

The plant hormone auxin (IAA) regulates many aspects of plant growth and development. Small auxin up RNA (SAUR) genes are highly transcribed in response to auxin, and have been implicated to function as a positive regulator of cellular expansion. *Arabidopsis* contains 79 SAUR genes within its genome. Current models predict SAUR proteins inhibit the PP2C.D clade of phosphatases, which normally dephosphorylate (inactivate) plasma membrane proton pumps. This phosphatase inhibition by SAURs allow active PM proton pumps, which are thought to cause cellular expansion. Using CRISPR-Cas9 technology, I aim to create 10 knockouts from the SAUR19 subfamily to confirm SAUR function. Using this loss-of-function approach, I hope to show reduced cellular expansion in a variety of auxin-related phenotypic assays.

Auxin Responsive Genes

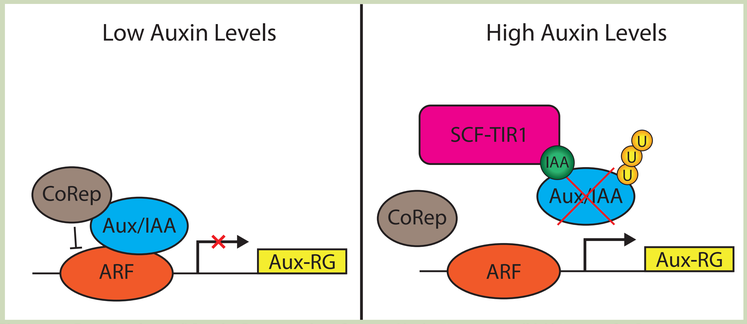


Figure 1. Regulation of Auxin Responsive Genes. Under low auxin (IAA) levels, Co-Repressors (CoRep) and Aux/IAA proteins bind to Auxin Response Factors (ARFs), preventing Auxin-Responsive genes (Aux-RG) from being transcribed. When auxin is present at high levels, it facilitates degradation of Aux/IAA proteins via a SCF-TIR1 ubiquitination complex. Degradation of AUX/IAA proteins releases Co-Repressors and allows transcription.

SAUR Function

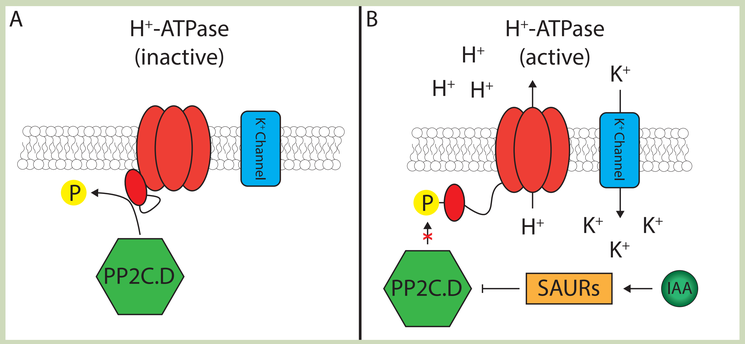


Figure 2. SAUR Function in Plasma Membrane Proton Pump Regulation. A) Without auxin (IAA) induced transcription of SAURs, PP2C.D clade phosphatases inactivate H^+-ATPases by removal of a regulatory phosphate group. **B)** When auxin is present, SAUR genes are upregulated and inhibit PP2C.D phosphatase activity. This allows the H^+-ATPases to function, pumping H^+ ions out of the cell. The resulting membrane hyperpolarization activates K^+ channels, promoting K^+ influx. To balance solute concentration, water enters the cell through osmosis, increasing the turgor pressure inside.

Cas9 Integration

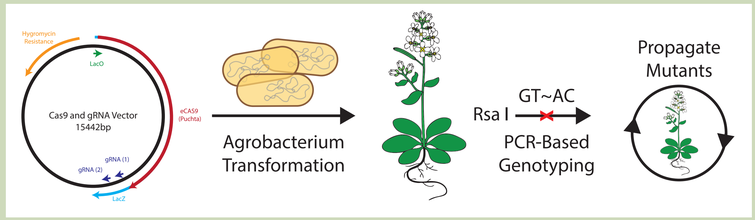


Figure 3. Cas9 Integration. A custom Cas9 and guide RNA vector is replicated with *E. coli* before being transferred to *A. tumefaciens*. These bacteria are then used to transform wild-type *Arabidopsis*. These plants are then genotyped and identified mutants are propagated. The mutant I assayed had homozygous knockouts of SAUR 19, 20, 21, 22, 24, and 29.

Target Gene Alignment

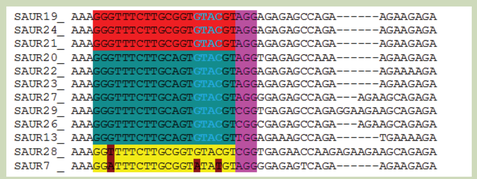
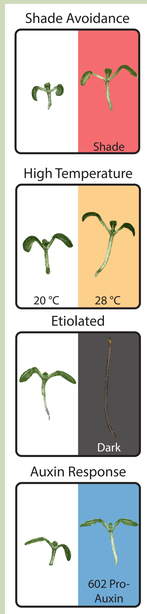


Figure 4. Target Alignment. Red - gRNA for S19, 24, and 21. Turquoise - gRNA for S20, 22, 23, 27, 29, 26, and 13. Yellow - denotes potential off-target matching of S28 and S7. Magenta - PAM site, cuts initiated 4bp upstream. Cyan - Rsa I digest site, mutations disrupt and allow for simple screening.

Assay



1. Shade Avoidance Response.
When plants are under shade conditions, simulated by the addition of far-red light, they elongate their hypocotyls to escape the shade. SAUR mutant plants could have a decreased shade avoidance response.

2. High Temperature Response.
When plants are grown in high temperature conditions, the biosynthesis of auxin increases. This in turn promotes expression of auxin-inducible genes. If SAUR mutant plants are deficient in this response, they should produce shorter hypocotyls.

3. Etiolated Grown Seedlings.
When grown in the dark, seedlings elongate their hypocotyls in an effort to reach the light. Normally this process is induced when plants are buried underground. SAUR mutant plants could be deficient in this response, producing shorter hypocotyls.

4. Exogenous Auxin Treatment.
The addition of a synthetic auxin precursor (602 Pro-auxin) has been shown to promote hypocotyl elongation in wild-type plants. SAUR mutant plants could be deficient in this response, producing shorter hypocotyls.

GUS Staining

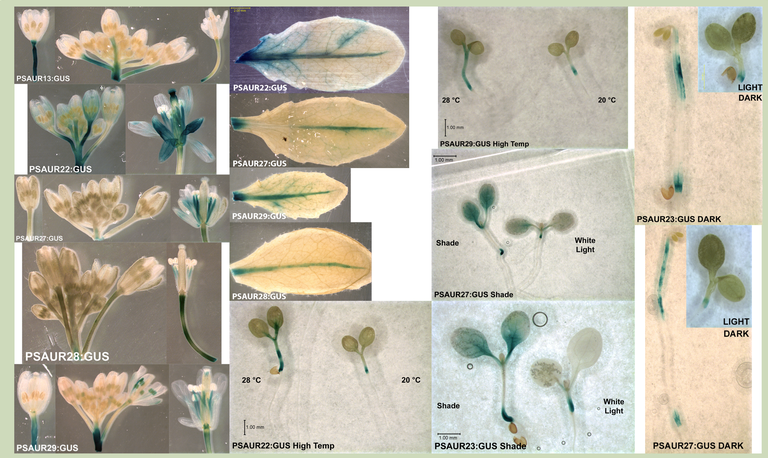


Figure 5. GUS staining. To Determine the location of SAUR gene expression, promoter-GUS constructs were created. This experiment reveals potential areas where SAUR defects could be detected. In adult leaf staining (far left), most SAUR expression was localized to leaf vasculature. In young flowers, some SAURs were expressed in stems, and most SAURs also were expressed in rapidly-expanding stamen filaments. Under dark (etiolated) conditions, most SAURs were highly expressed in the elongation zone (top 1/3) of the hypocotyl. In both high temperature and shade conditions, most SAURs were expressed in hypocotyls and petioles, with some SAURs being expressed in leaves and in root vasculature.

Results

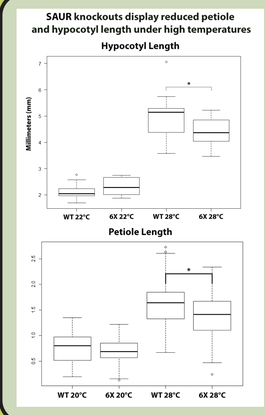


Figure 6. High Temperature Growth Defect. SAUR sextuple (6X) mutants containing SAURs 19, 20, 21, 22, 24, and 29 exhibited reduced growth of hypocotyls ($p=0.02$) and petioles ($p=0.02$) when under high temperature (28 °C).

No Figure. No Significant Difference in Shade, Etiolated, or Auxin Treatment Hypocotyl Lengths. While not pictured, there was no significant differences in hypocotyl length in our other assayed conditions. This may suggest that the SAUR19 subfamily could play a larger role in temperature-related growth than the other assayed conditions. Alternatively, these negative results support the theory of extensive genetic redundancy within the SAUR gene family.

Discussion

In my thesis work, I have shown supportive evidence that SAUR genes are positive regulators of cellular expansion. In addition to investigating unknown expression patterns of SAUR 13,22, 27, 28, and 29, my project provided encouraging evidence that SAURs are important for high temperature induced hypocotyl and petiole elongation. This is the largest combination of SAUR gene knockouts to date. While the sextuple mutant I helped to generate displays some auxin-related cell expansion defects, they are inconsistent across different types of assays.